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(54) Title: LSR, A CDNA OF THE IG SUPERFAMILY EXPRESSED IN HUMAN LEUKOCYTES

(57) Abstract: The invention is directed to purified and isolated LSR polypeptides, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and the uses of the above.

LSR, A CDNA OF THE IG SUPERFAMILY EXPRESSED IN HUMAN LEUKOCYTES

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to purified and isolated novel Leukocyte Surface Receptor (LSR) polypeptides and fragments thereof, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

Description of Related Art

Immune system cellular activity is controlled by a complex network of cell surface interactions and associated signaling processes. When a cell surface receptor is activated by its ligand, a signal is sent to the cell. Depending upon the signal transduction pathway that is engaged, the signal can be inhibitory or activatory.

Immune system activatory and inhibitory signals mediated by opposing kinases and phosphatases are very important for maintaining balance in the immune system. Systems with a predominance of activatory signals will lead to autoimmunity and inflammation. Immune systems with a predominance of inhibitory signals are in a compromised position to challenge infected cells or cancer cells. Isolating new activatory or inhibitory receptors is highly desirable for studying the biological signal(s) transduced via the receptor. Additionally, identifying such molecules provides a means of regulating and treating diseased states associated with autoimmunity, inflammation and infection.

The Immunoglobulin ("Ig") superfamily of molecules is one of the key groups not only in immunity but also in the mediation of cell surface recognition to control the behavior of cells in various tissues. (Williams et al., *Ann. Rev. Immunol.* *6*:381-405, 1988) The structural basis for the Ig superfamily rests on gene duplication and divergence of domains of about 100 amino acids that show a characteristic folding pattern to give a structure with two β sheets stabilized by a hydrophobic interior and often by a characteristic disulfide bind

between the sheets. (Williams et al., *Cold Spring Harbor Symposia on Quantitative Biology*, Vol. LIV, 637-47, 1989). Members of the Ig superfamily include Immunoglobulins, T cell receptor complex, Major histocompatibility complexes (MHC antigens), β_2-m associated antigens, T cell adhesion molecules, T subset antigens, Brain/lymphoid antigens, Ig receptors, tumor antigen and growth factor receptors. (Williams et al., *Ann. Rev. Immunol.* *6*:381-405, Table 1, 1988)

Given the important and varied functions of the Ig superfamily members, there is a need in the art to identify additional members of this family. Further, in view of the continuing interest in protein research and the immune system, the discovery, identification, and roles of new proteins (such as the human LSR of the invention) and their inhibitors, are at the forefront of modem molecular biology and biochemistry. Despite the growing body of knowledge, there is still a need in the art for the identity and function of proteins involved in cellular and immune responses.

In another aspect, the identification of the primary structure, or sequence, of an unknown protein is the culmination of an arduous process of experimentation. In order to identify an unknown protein, the investigator can rely upon a comparison of the unknown protein to known peptides using a variety of techniques known to those skilled in those skilled in the art. For instance, proteins are routinely analyzed using techniques such as electrophoresis, sedimentation, chromatography, sequencing and mass spectrometry.

In particular, comparison of an unknown protein to polypeptides of known molecular weight allows a determination of the apparent molecular weight of the unknown protein (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77, Prentice Hall, 6d ed., 1991). Protein molecular weight standards are commercially available to assist in the estimation of molecular weights of unknown protein (New England Biolabs Inc. Catalog: 130-131, 1995; J. L. Hartley, U.S. Patent No. 5,449,758). However, the molecular weight standards may not correspond closely enough in size to the unknown protein to allow an accurate estimation of apparent molecular weight. The difficulty in estimation of molecular weight is compounded in the case of proteins that are subjected to fragmentation by chemical or enzymatic means, modified by post-translational modification or processing, and/or associated with other proteins in non-covalent complexes.

In addition, the unique nature of the composition of a protein with regard to its specific amino acid constituents results in unique positioning of cleavage sites within the protein. Specific fragmentation of a protein by chemical or enzymatic cleavage results in a

unique "peptide fingerprint" (D.W. Cleveland et al., *J. Biol. Chem. 252*:1102-1106, 1977; M. Brown et al., *J. Gen. Virol. 50*:309-316, 1980). Consequently, cleavage at specific sites results in reproducible fragmentation of a given protein into peptides of precise molecular weights. Furthermore, these peptides possess unique charge characteristics that determine the isoelectric pH of the peptide. These unique characteristics can be exploited using a variety of electrophoretic and other techniques (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77, Prentice Hall, 6d ed., 1991).

Fragmentation of proteins is further employed for amino acid composition analysis and protein sequencing (P. Matsudiara, *J. Biol. Chem. 262*:10035-10038, 1987; C. Eckerskom et al., *Electrophoresis 9*:830-838, 1988), particularly the production of fragments from proteins with a "blocked" N-terminus. In addition, fragmented proteins can be used for immunization, for affinity selection (R. A. Brown, U.S. Patent No. 5,151,412), for determination of modification sites (*e.g.* phosphorylation), for generation of active biological compounds (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 300-301, Prentice Hall, 6d ed., 1991), and for differentiation of homologous proteins (M. Brown et al., *J. Gen. Virol. 50*:309-316, 1980).

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA 90*:5011-5015, 1993; D. Fenyo et al., *Electrophoresis 19*:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site:prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

Furthermore, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.* *5*:976-989 (1994); M. Mann and Wilm, *Anal. Chem.* *66*:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.* 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

Thus, there also exists a need in the art for polypeptides suitable for use in peptide fragmentation studies, for use in molecular weight measurements, and for use in protein sequencing using tandem mass spectrometry. Moreover, the nucleic acids of the present invention are useful for chromosome identification. Each of the disclosed nucleic acids will hybridize with a particular location on an individual human chromosome, and thereafter can be correlated with diseases that are associated with that same chromosome locus (see, e.g., V. McKusick, Mendelian Inheritance in Man, available at the Johns Hopkins University Medical Library website).

SUMMARY OF THE INVENTION

The invention aids in fulfilling these various needs in the art by providing isolated nucleic acids and polypeptides encoded by these nucleic acids for a novel immunoglobulin family protein termed Leukocyte Surface Receptor (LSR). Particular embodiments of the invention are directed to isolated LSR nucleic acid molecules comprising the DNA sequences of SEQ ID NOS:1 and 3 and isolated LSR nucleic acid molecules encoding the amino acid sequences of SEQ ID NOS:2 and 4, as well as nucleic acid molecules complementary to these sequences.

Both single-stranded and double-stranded RNA and DNA nucleic acid molecules are encompassed by the invention, as well as nucleic acid molecules that hybridize to a denatured, double-stranded DNA comprising all or selected portions of SEQ ID NOS:1 or 3 and/or DNA that encodes the amino acid sequences set forth in SEQ ID NOS:2 or 4. Also encompassed are isolated nucleic acid molecules that are derived by *in vitro* mutagenesis of nucleic acid molecules comprising sequences of SEQ ID NOS:1 and 3, that are degenerate from nucleic acid molecules comprising sequences of SEQ ID NOS:1 and 3, and that are

allelic variants of DNA of the invention. The invention also encompasses recombinant vectors that direct the expression of these nucleic acid molecules and host cells stably or transiently transformed or transfected with these vectors.

In addition, the invention encompasses methods of using any of these nucleic acid molecules to identify nucleic acids encoding proteins having the activity of the Ig superfamily. Further, the invention encompasses methods to identify human chromosome number 17; to map genes on human chromosome number 17 and to identify genes associated with certain diseases, syndromes, or other human conditions associated with human chromosome number 17.

The invention also encompasses the use of sense or antisense oligonucleotides from the nucleic acid of SEQ ID NOS:1 and 3 to inhibit the expression of the polynucleotide encoded by the genes of the instant invention.

The invention also encompasses isolated polypeptides and fragments thereof encoded by the nucleic acid molecules noted above. The invention further encompasses methods for the production of these polypeptides, including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of these polypeptides in bacteria, yeast, plant, insect, and animal cells is encompassed by the invention.

In general, the polypeptides of the invention can be used to study cellular processes such as immune regulation, cell proliferation, cell death, cell migration, cell-to-cell interaction, and inflammatory responses. In addition, these polypeptides can be used to identify proteins associated with Ig proteins.

In addition, the invention includes assays utilizing these polypeptides to screen or potential inhibitors of activity associated with polypeptide counterstructure molecules, and methods of using these polypeptides as therapeutic agents for the treatment of diseases mediated by LSR polypeptide counterstructure molecules. Further, methods of using these polypeptides in the design of inhibitors thereof are also an aspect of the invention.

The invention further provides a method for using the LSR polypeptides as molecular weight markers that allow the estimation of the molecular weight of a protein or a fragmented protein, as well as a method for the visualization of the molecular weight markers of the invention thereof using electrophoresis. The invention further encompasses methods for using the polypeptides of the invention as markers for determining the isoelectric point of an unknown protein, as well as controls for establishing the extent of fragmentation of a protein.

Further encompassed by this invention are kits to aid in these determinations.

Still further encompassed by this invention is the use of the LSR nucleic acid sequences, predicted amino acid sequences of the polypeptide or fragments thereof, or a combination of the predicted amino acid sequences of the polypeptide and thereof for use in searching an electronic database to aid in the identification of sample nucleic acids and/or proteins.

Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are also encompassed by the invention, in addition the use of these antibodies to aid in purifying the LSR polypeptide.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 presents the nucleotide sequence of an embodiment of LSR (SEQ ID NO:1).

Figure 2 presents the amino acid sequence of an embodiment of LSR (SEQ ID NO:2).

Figure 3 presents the nucleotide sequence of an embodiment of LSR (SEQ ID NO:3).

Figure 4 presents the amino acid sequence of an embodiment of LSR (SEQ ID NO:4).

DETAILED DESCRIPTION OF THE INVENTION

The nucleic acid molecules encompassed in the invention include the following nucleotide sequence:

```
  1 TGTCAATAAC CCCTCCTAGT TTGGGTGTTC CCATAGGCTG AGGGATGCCC
 51 CTCCTGTACC CTTGGGGGAC ATAACAGTAT CTAATAAATT TCAAAGAGAA
101 ATGCCAGCGG CCGCTGAATT CTAGCTGGGA TCTGCATTTG CCACTGGTTG
151 CAGATCAGGC GGACGAGGAG CCGGGAAGGC AGAGCCATGT GGCTGCCCCC
201 TGCTCTGCTC CTTCTCAGCC TCTCAGGCTG TTTCTCCATCAAGGCCCAG
251 AGTCTGTGAG AGCCCCAGAG CAGGGGTCCC TGACGGTTCAATGCCACTAT
301 AAGCAAGGAT GGGAGACCTA CATTAAGTGG TGGTGCCGAG GGGTGCGCTG
351 GGATACATGC AAGATCCTCA TTGAAACCAG AGGGTCGGAG CAAGGAGAGA
401 AGAGTGACCG TGTGTCCATC AAGGACAATC AGAAAGACCG CACGTTCACT
451 GTGACCATGG AGGGGCTCAC GCGAGATGAC GCAGATGTTT ACTGGTGTGG
501 GATTGAAAGA AGAGGACCTG ACCTTGGGAC TCAAGTGAAA GTGATTGTTG
551 ACCCAGAGGG AGCGGCTTCC ACAACAGCAA GCTCACCTAC CAACAGCAAT
601 ATGGCAGTGT TCATCGGCTC CCACAAGAGG AACCACTACA TGCTCCTGGT
651 ATTTGTGAAG GTGCCCATCT TGCTCATCTT GGTCACTGCC ATCCTCTGGT
701 TGAAGGGGTC TCAGAGGGTC CCTGAGGAGC CAGGGGAACA GCCTATCTAC
751 ATGAACTTCT CCGAACCTCT GACTAAAGAC ATGGCCACTT AGAGAGATGG
801 ATCTGCAGAG CCTTCCTGCC CTGGCCACGT TTCCAGAAGA GACTCGGGCT
851 GT (SEQ ID NO:1)
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In another embodiment, the nucleic acid molecules encompassed in the invention include the following nucleotide sequence:

```
  1 TGTCAATAAC CCCTCCTAGT TTGGGTGTTC CCATAGGCTG AGGGATGCCC
 51 CTCCTGTACC CTTGGGGGAC ATAACAGTAT CTAATAAATT TCAAAGAGAA
101 ATGCCAGCGG CCGCTGAATT CTAGCTGGGA TCTGCATTTG CCACTGGTTG
151 CAGATCAGGC GGACGAGGAG CCGGGAAGGC AGAGCCATGT GGCTGCCCCC
201 TGCTCTGCTC CTTCTCAGCC TCTCAGGCTG TTTCTCCATC CAAGGCCCAG
251 AGTCTGTGAG AGCCCCAGAG CAGGGGTCCC TGACGGTTCA ATGCCACTAT
301 AAGCAAGGAT GGGAGACCTA CATTAAGTGG TGGTGCCGAG GGGTGCGCTG
351 GGATACATGC AAGATCCTCA TTGAAACCAG AGGGTCGGAG CAAGGAGAGA
401 AGAGTGACCG TGTGTCCATC AAGGACAATC AGAAAGACCG CACGTTCACT
451 GTGACCATGG AGGGGCTCAC GCGAGATGAC GCAGATGTTT ACTGGTGTGG
501 GATTGAAAGA AGAGGACCTG ACCTTGGGAC TCAAGTGAAA GTGATTGTTG
551 ACCCAGAGGG AGCGGCTTCC ACAACAGCAA GCTCACCTAC CAACAGCAAT
601 ATGGCAGTGT TCATCGGCTC CCACAAGAGG AACCACTACA TGCTCCTGGT
651 ATTTGTGAAG GTGCCCATCT TGCTCATCTT GGTCACTGCC ATCCTCTGGT
701 TGAAGGGGTC TCAGAGGGTC CCTGAGGAGC CAGGGGAACA GCCTATCTAC
751 ATGAACTTCT CCGAACCTCT GACTAAAGAC ATGGCCACTT AGAGAGATGG
801 ATCTGCAGAG CCTTCCTGCC CTGGCCACGT TTCCAGAAGA GACTCGGGCT
851 GT (SEQ ID NO:3)
```

The amino acid sequence of the polypeptide encoded by the nucleotide sequence of the invention includes:

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  1 MWLPPALLLL SLSGCFSIQG PESVRAPEQG SLTVQCHYKQ GWETYIKWWC
 51 RGVRWDTCKI LIETRCSEQG EKSDRVSIKD NQKDRTFTVT MEGLTRDDAD
101 VYWCGIERRG PDLGTQVKVI VDPEGAASTT ASSPTNSNMA VFIGSHKRNH
151 YMLLVFVKVP ILLILVTAIL WLKGSQRVPE EPGEQPIYMN FSEPLTKDMA
201 T (SEQ ID NO:2)
```

In another embodiment, the amino acids encompassed in the invention include the following sequence:

```
  1 MWLPPALLLL SLSGCFSIQG PESVRAPEQG SLTVQCHYKQ GWETYIKWWC
 51 RGVRWDTCKI LIETRGSEQG EKSDRVSIKD NQKDRTFTVT MEGLTRDDAD
101 VYWCGIERRG PDLGTQVKVI VDPEGAASTT ASSPTNSNMA VFIGSHKRNH
151 YMLLVFVKVP ILLILVTAIL WLKGSQRVPE EPGEQPIYMN FSEPLTKDMA
201 T(SEQ ID NO:4)
```

The discovery of the nucleic acids of the invention enables the construction of expression vectors comprising nucleic acid sequences encoding polypeptides, host cells

transfected or transformed with the expression vectors, and isolated and purified biologically active polypeptides and fragments thereof. In yet another embodiment, the use of the nucleic acids or oligonucleotides thereof can be used as probes to identify nucleic acid encoding proteins having activities associated with immunoglobulin proteins. The single-stranded sense or antisense oligonucleotides from the nucleic acids can also be used to inhibit expression of polynucleotide encoded by the LSR gene. Such polypeptides and fragmented peptides can be used as molecular weight markers and as controls for peptide fragmentation, and kits comprising these reagents. The LSR polypeptides and fragments thereof can also be used to generate antibodies. Antibodies of LSR can be used to purify the LSR polypeptide.

In a particular embodiment, the invention provides isolated nucleic molecules that are free from contaminating endogenous material. A "nucleic acid molecule" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecules of the invention are derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or construction enabling their identification, manipulation, and recovery by standard biochemical methods (such as those outlined in Sambrook et al., 1989). Such nucleic acid molecules are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA may be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

PREFERRED NUCLEIC ACID MOLECULES

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, *e.g.*, using the cDNA of SEQ ID NOS:1 or 3, or a suitable fragment thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may include the sequence of the N-terminal signal peptide.

The nucleic acids of the invention are preferentially derived from human sources, but the invention includes those derived from non-human species, as well.

Preferred Sequences

A particularly preferred nucleotide sequence of the invention is SEQ ID NO:1 and an even more preferred sequence is SEQ ID NO:3, as set forth above. cDNA clones having the nucleotide sequences of SEQ ID NOS:1 or 3 were isolated as described in Example 1. The sequences of amino acids encoded by the DNA of SEQ ID NOS:1 and 3 are shown in SEQ ID NOS:2 and 4, respectively. These sequences identify the LSR polynucleotide as a member of the Ig family.

Additional Sequences

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:3, for example, and still encode a polypeptide having the amino acid sequence of SEQ ID NO:4. Such variant DNA sequences can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences encoding polypeptides of the invention, selected from: (a) DNA comprising the nucleotide sequences of SEQ ID NOS:1 or 3; (b) DNA encoding the polypeptides of SEQ ID NOS:2 or 4; (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate stringency and which encodes polypeptides of the invention; (d) DNA capable of hybridization to a DNA of (a) or (b) under conditions of high stringency and which encodes polypeptides of the invention, and (e) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b), (c), or (d) and which encodes polypeptides of the invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention.

The invention thus provides isolated nucleic acid molecules encoding polypeptides of the invention, selected from: (a) a nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1 or 3; (b) a nucleic acid molecule encoding the polypeptides of SEQ ID NOS:2 or 4; (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate stringency and which encodes a polypeptide of the invention; (d) DNA capable of hybridization to a DNA of (a) or (b) under conditions of high stringency and which encodes a polypeptide of the invention, and (e) DNA or RNA that is degenerate as a result of the genetic code to a DNA defined in (a), (b), (c), or (d) and which encodes a polypeptide of the invention. In addition, polypeptides themselves encoded by such nucleic acid sequences are encompassed by the invention.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook et al., *Molecular Cloning: A Laboratory Manual* 2.ed, Vol. 1, pp. 1. 101-104 (1989). For hybridizing probes longer than about 100 nucleotides with filter-bound target DNA or RNA, one way of achieving moderately stringent conditions involves the use of a prewashing solution containing 5 x SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6 x SSC, and a hybridization temperature of about 42°C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of about 42°C), and washing conditions of about 60°C, in 0.5 x SSC, 0.1% SDS. Conditions of high stringency can also be readily determined by the skilled artisan based on, for example, the length and base composition of the DNA. Generally, such conditions are defined as hybridization conditions as above, but with washing at approximately 68°C, 0.2 x SSC, 0.1% SDS. The skilled artisan will recognize that the wash temperature and wash salt concentration can be adjusted as necessary to achieve a desired degree of stringency by applying the basic principles that govern hybridization reactions and duplex stability, as known to those skilled in the art (*e.g.*, see Sambrook et al., 1989). It should be further understood that hybridization conditions for oligonucleotide probes of defined length and sequence can be designed by applying formulae known in the art (*e.g.*, see Sambrook et al., 1989, at 11.45-11.47).

Also included as an embodiment of the invention is DNA encoding polypeptide fragments and polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or conservative amino acid substitution(s), as described below.

In another embodiment, the nucleic acid molecules of the invention also comprise nucleotide sequences that are at least 80% identical to a native sequence. Also contemplated are embodiments in which a nucleic acid molecule comprises a sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a native sequence.

Percent identity may be determined by visual inspection. Percent identity may be determined using the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970) as revised by Smith and Waterman (Adv. Appl. Math 2:482, 1981). Preferably, percent identity is determined by using a computer program, for example, the GAP computer

program version 10.x available from the Genetics Computer Group (GCG; Madison, WI, see also Devereux et al., *Nucl. Acids Res.* 12:387, 1984). The preferred default parameters for the GAP program include: (1) a comparison matrix containing a value of 1 for identities and 0 for non-identities for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979 for amino acids; (2) a penalty of 30 (amino acids) or 50 (nucleotides) for each gap and an additional 1 (amino acids) or 3 (nucleotides) penalty for each symbol in each gap; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding the polypeptide of the invention, or desired fragment thereof may be subcloned into an expression vector for production of the polypeptide or fragment. The DNA sequence advantageously is fused to a sequence encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5'or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA sequence encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science 239*:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

Polypeptides and Fragments Thereof

The polypeptides of the invention include full length proteins encoded by the nucleic acid sequences set forth above. Particularly preferred polypeptides comprise the amino acid sequence of SEQ ID NO:2. More preferred polypeptides comprise the amino acid sequence of SEQ ID NO:4.

The polypeptide of SEQ ID NO:4, for example, includes an N-terminal hydrophobic region that functions as a signal peptide. Computer analysis predicts that the signal peptide corresponds to residues 1 to 18 of SEQ ID NO:4. Although the exact location of the extracellular, transmembrane, and cytoplasmic domains may differ slightly due to different analytical criteria for identifying the functional domains, the extracellular domain of the novel polypeptide generally comprises amino acids 1 to 151; the transmembrane region comprises amino acids 152 through 172, and the cytoplasmic domain comprises amino acids 173 to 201. A spacer region comprises amino acids 122 to 151. Cleavage of the signal peptide thus would yield a mature protein comprising amino acids 19 through 201 of SEQ ID NO:4.

The skilled artisan will recognize that the above-described boundaries of such regions of the polypeptide are approximate and that the boundaries of the transmembrane region (which may be predicted by using computer programs available for that purpose) may differ from those described above.

Particular embodiments of the invention are directed to polypeptide fragments that retain the ability to bind the native cognates, substrates, or counterstructure ("binding partner"). In another embodiment, the polypeptides and fragments advantageously include regions that are conserved in the Ig family as described above.

Also provided herein are polypeptide fragments comprising at least 20, or at least 30, contiguous amino acids of the sequences of SEQ ID NOS:2 or 4. Fragments derived from the cytoplasmic domain find use in studies of signal transduction, and in regulating cellular

processes associated with transduction of biological signals. Polypeptide fragments also may be employed as immunogens, in generating antibodies.

Variants

Naturally occurring variants, as well as derived variants of the polypeptides and fragments, are provided herein. Variants may exhibit amino acid sequences that are at least 80% identical. Also contemplated are embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence information using the GAP computer program, based on the algorithm of Needleman and Wunsch (*J. Mol. Bio. 48*:443, 1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff (*Proc. Natl. Acad. Sci. USA 89*:10915, 1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Additional variants within the scope of the invention include polypeptides that may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared by linking the chemical moieties to functional groups on amino acid side chains or at the N-terminus or C-terminus of a polypeptide.

Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are contemplated herein, as discussed in more detail below.

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Among the variant polypeptides provided herein are variants of native polypeptides that retain the native biological activity or the substantial equivalent thereof. One example is a variant that binds with essentially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, *e.g.*, as described in U.S. Patent No. 5,512,457, and as set forth below.

Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for

one another. Other conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

The invention further includes polypeptides of the invention with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) can be similar to or significantly different from a native polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, a given preparation may include multiple differentially glycosylated species of the protein. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Oligomers

Also encompassed by the invention are oligomers or fusion proteins that contain LSR polypeptides. The polypeptide of the instant invention is a Type 1 membrane protein. It is therefore preferable for the fusion partner to be linked to the C-terminus of the Type 1 membrane protein. Such oligomers may be in the form of covalently-linked or non-

covalently-linked multimers, including dimers, trimers, or higher oligomers. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites.

One embodiment of the invention is directed to oligomers comprising multiple polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of the polypeptides attached thereto, as described in more detail below.

Immunoglobulin-based Oligomers

As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al. (*PNAS USA 88*:10535, 1991); Byrn et al. (*Nature 344*:677, 1990); and Hollenbaugh and Aruffo, "Construction of Immunoglobulin Fusion Proteins," in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992.

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion protein is inserted into an appropriate expression vector. Polypeptide/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent molecules.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. Preferred polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful

Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al. (*EMBO J. 13*:3992-4001, 1994), incorporated herein by reference. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

In other embodiments, the polypeptides of the invention may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four polypeptide extracellular regions.

Alternatively, the oligomer is a fusion protein comprising multiple polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between the sequences.

Leucine-Zippers

Another method for preparing the oligomers of the invention involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science 240*:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize.

The zipper domain (also referred to herein as an oligomerizing, or oligomer- forming, domain) comprises a repetitive heptad repeat, often with four or five leucine residues interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science 243*:1681, 1989). Two nuclear transforming proteins, *fos*

and *jun*, also exhibit zipper domains, as does the gene product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form heterodimer (OShea et al., *Science 245*:646, 1989, Turner and Tjian, *Science 243*:1689, 1989). The zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess zipper domains (Buckland and Wild, *Nature 338*:547,1989; Britton, *Nature 353*:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses 6*:703, 1990). The zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. USA 88*:3523, 1991). Zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science 259*:230, 1993).

Zipper domains fold as short, parallel coiled coils (OShea et al., *Science 254*:539; 1991). The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr. 6*:689). The dimer formed by a zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol. 98*:293; 1975), in which residues *a* and *d* are generally hydrophobic residues, with *d* being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The residues at position *d* (often leucine) contribute large hydrophobic stabilization energies, and are important for oligomer formation (Krystek: et al., *Int. J. Peptide Res. 38*:229, 1991). Lovejoy et al. (*Science 259*:1288, 1993) recently reported the synthesis of a triple-stranded α-helical bundle in which the helices run up-up-down. Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils.

Further discussion of the structure of leucine zippers is found in Harbury et al (*Science* *262*:1401, 26 November 1993).

Certain leucine zipper moieties preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD) noted above, as described in Hoppe et al. (*FEBS Letters 344*:191, 1994) and in U.S. Patent 5,716,805, hereby incorporated by reference in their entirety. This lung SPD-derived leucine zipper peptide comprises the amino acid sequence Pro Asp Val Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr.

Another example of a leucine zipper that promotes trimerization is a peptide comprising the amino acid sequence Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg, as described in U.S. Patent 5,716,805. In one alternative embodiment, an N-terminal Asp residue is added; in another, the peptide lacks the N-terminal Arg residue.

Fragments of the foregoing zipper peptides that retain the property of promoting oligomerization may be employed as well. Examples of such fragments include, but are not limited to, peptides lacking one or two of the N-terminal or C- terminal residues presented in the foregoing amino acid sequences. Leucine zippers may be derived from naturally occurring leucine zipper peptides, *e.g.*, via conservative substitutions in the native amino acid sequence, wherein the peptide's ability to promote oligomerization is retained.

Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric oligomers. Alternatively, synthetic peptides that promote oligomerization may be employed. In particular embodiments, leucine residues in a leucine zipper moiety are replaced by isoleucine residues. Such peptides comprising isoleucine may be referred to as isoleucine zippers, but are encompassed by the term "leucine zippers" as employed herein.

PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF

Expression, isolation and purification of the polypeptides and fragments of the invention may be accomplished by any suitable technique, including but not limited to the following:

Expression Systems

The present invention also provides recombinant cloning and expression vectors containing DNA, as well as host cells containing the recombinant vectors. Expression

vectors comprising DNA may be used to prepare the polypeptides or fragments of the invention encoded by the DNA. A method for producing polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding the polypeptide, under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed.

Any suitable expression system may be employed. The vectors include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site. Particular embodiments of mature proteins provided herein include, but are not limited to, proteins having the residue at position 19 of SEQ ID NOS:2 or 4 as the N-terminal amino acid.

Suitable host cells for expression of polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotic Systems

Prokaryotes include gram-negative or gram-positive organisms. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, a polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N- terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and PGEMI (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β-lactamase (penicillinase), lactose promoter system (Chang et al., *Nature* *275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EP-A-36776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λP_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American

Type Culture Collection which incorporate derivatives of the λP_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC 53082).

Yeast Systems

Alternatively, the polypeptides may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (*e.g., S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149, 1968; and Holland et al., *Biochem. 17*:4900, 1978), such as enolase, glyceraidehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6- phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem. 258*:2674, 1982) and Beier et al. (*Nature 300*:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of the polypeptide. The (α-factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. See, *e.g.*, Kurjan et al., *Cell* 30:933, 1982, and Bitter et al., *Proc. Natl Acad. Sci. USA 81*:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA 75*:1929, 1978. The Hinnen et al. protocol selects for Trp^+ transformants in a selective medium, wherein the

selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mg/ml adenine and 20 mg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 mg/ml adenine and 80 mg/ml uracil. Depression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or Insect Systems

Mammalian or insect host cell culture systems also may be employed to express recombinant polypeptides. Bacculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology 6*:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell 23*:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CVI/EBNA cell line derived from the African Green Monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J. 10*: 2821, 1991).

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture,* 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA 84*:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2 ed., Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology 185*:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector

include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al., *Nature 273*:113, 1978; Kaufman, *Meth. in Enzymology*, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, 1997, pp. 529-534 and PCT Application WO 97/25420) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem. 257*:13475-13491, 1982). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development 3*:295-300, 1993; Ramesh et al., *Nucleic Acids Research 24*:2697-2700, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (*e.g.* DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman, *Meth. in Enzymology*, 1990). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques 22*:150-161, 1997, and p2A51 described by Morris et al., *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley et al., *Cell 59*:335-348, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol. 3*:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.*

23:935, 1986). A useful high expression vector, PMLSV Nl/N4, described by Cosman et al., *Nature 312*:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982, incorporated by reference herein. In yet another alternative, the vectors can be derived from retroviruses.

Additional useful expression vectors, pFLAG® and pDC311, can also be used. FLAG® technology is centered on the fusion of a low molecular weight (lkD), hydrophilic, FLAG® marker peptide to the N-terminus or C-terminus of a recombinant protein expressed by pFLAG® expression vectors. pDC311 is another specialized vector used for expressing proteins in CHO cells. pDC311 is characterized by a bicistronic sequence containing the gene of interest and a dihydrofolate reductase (DHFR) gene with an internal ribosome binding site for DHFR translation, an expression augmenting sequence element (EASE), the human CMV promoter, a tripartite leader sequence, and a polyadenylation site.

Regarding signal peptides that may be employed, the native signal peptide may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature 312*:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Purification

The invention also includes methods of isolating and purifying the polypeptides and fragments thereof.

Isolation and Purification

The "isolated" polypeptides or fragments thereof encompassed by this invention are polypeptides or fragments that are not in an environment identical to an environment in which it or they can be found in nature. The "purified" polypeptides or fragments thereof encompassed by this invention are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant expression systems such

as those described above or as a purified product from a non-recombinant source such as naturally occurring cells and/or tissues.

In one preferred embodiment, the purification of recombinant polypeptides or fragments can be accomplished using fusions of polypeptides or fragments of the invention to another polypeptide to aid in the purification of polypeptides or fragments of the invention. Such fusion partners can include the poly-His or other antigenic identification peptides described above as well as the Fc moieties described previously.

With respect to any type of host cell, as is known to the skilled artisan, procedures for purifying a recombinant polypeptide or fragment will vary according to such factors as the type of host cells employed and whether or not the recombinant polypeptide or fragment is secreted into the culture medium.

In general, the recombinant polypeptide or fragment can be isolated from the host cells if not secreted, followed by one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification or size exclusion chromatography steps. As to specific ways to accomplish these steps, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In addition, a chromatofocusing step can be employed. Alternatively, a hydrophobic interaction chromatography step can be employed. Suitable matrices can be phenyl or octyl moieties bound to resins. In addition, affinity chromatography with a matrix which selectively binds the recombinant protein can be employed. Examples of such resins employed are lectin columns, dye columns, and metal-chelating columns. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (*e.g.*, silica get or polymer resin having pendant methyl, octyl, octyldecyl or other aliphatic groups) can be employed to further purify the polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

It is also possible to utilize an affinity column comprising a polypeptide-binding protein of the invention, such as a monoclonal antibody generated against polypeptides of the invention, to affinity-purify expressed polypeptides. These polypeptides can be removed from an affinity column using conventional techniques, *e.g.*, in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention.

In this aspect of the invention, polypeptide-binding proteins, such as the anti-polypeptide antibodies of the invention or other proteins that may interact with the polypeptide of the invention, can be bound to a solid phase support such as a column chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding proteins of the invention to a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with these polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has such polypeptide-binding proteins thereon. Cells having polypeptides of the invention on their surface bind to the fixed polypeptide-binding protein and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening, or separating such polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding protein of the invention. Incubation periods are typically at least one hour in duration to ensure sufficient binding to polypeptides of the invention. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See, Berenson et al., *J. Cell. Biochem.*, *10D*:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the polypeptide is to be administered *in vivo*, for example. In such a case, the polypeptides are purified such that no protein bands corresponding to other proteins are detectable upon analysis by SDS- polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

Assays

The purified polypeptides of the invention (including proteins, polypeptides, fragments, variants, oligomers, and other forms) may be tested for the ability to bind the binding partner in any suitable assay, such as a conventional binding assay. To illustrate, the polypeptide may be labeled with a detectable reagent (*e.g.*, a radionuclide, chromophore, enzyme that catalyzes a calorimetric or fluorometric reaction, and the like). The labeled polypeptide is contacted with cells expressing the binding partner. The cells then are washed to remove unbound labeled polypeptide, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

One example of a binding assay procedure is as follows. A recombinant expression vector containing the binding partner cDNA is constructed using methods well-known in the art. CV1-EBNA-1 cells (ATCC CRL 10478) in 10 cm^2 dishes are transfected with the recombinant expression vector. CV1-EBNA-1 cells constitutively express EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al. (*EMBO J. 10*:2821, 1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4 x 10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at

37°C with various concentrations of, for example, a polypeptide/Fc fusion protein made as set forth above. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any polypeptide/Fc protein that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed in the absence of the Fc fusion protein/Fc, as well as in the presence of the Fc fusion protein and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody.

Cell-bound ^{125}I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N. Y Acad. Sci. 51*:660, 1949) are generated on RS/I (BBN Software, Boston, MA) run on a Microvax computer.

The LSR polypeptide of the present invention may also be used as a screening assay for compounds and small molecules that activate (agonists) or inhibit activation of (antagonists) the LSR polypeptide of the instant invention. Thus, polypeptides of the invention may be used to identify agonists or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. The agonists and antagonists may be natural or modified substrates, ligands, enzymes, receptors, etc. of the LSR polypeptide, or may be structural or functional mimetics of the LSR polypeptide. The agonists and antagonists may further be small molecules, peptides, antibodies and antisense oligonucleotides.

One embodiment of a method for identifying compounds that antagonize or agonize the LSR polypeptide is contacting a candidate compound with cells that either express or respond to LSR polypeptide and observe the binding, or stimulation or inhibition of a functional response. The activity of the cells that were contacted with the candidate compound could then be compared with the identical cells that were not contacted for LSR polypeptide activity and LSR polypeptide agonists and antagonists could be identified.

USE OF LSR NUCLEIC ACID OR OLIGONUCLEOTIDES

In addition to being used to express polypeptides as described above, the nucleic acids of the invention, including DNA, and oligonucleotides thereof can be used:

- as probes to identify nucleic acid encoding proteins having activity associated with the immunoglobulin family;
- to identify human chromosome number 17;
- to map genes on human chromosome number 17;
- to identify genes associated with certain diseases, syndromes, or other conditions associated with human chromosome number 17;
- as single-stranded sense or antisense oligonucleotides, to inhibit expression of polypeptide encoded by the LSR gene;
- to help detect defective genes in an individual; and
- for gene therapy.

Probes

Among the uses of nucleic acids of the invention is the use of fragments as probes or primers. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a DNA sequence.

Because homologs of SEQ ID NO:1 and 3 from other mammalian species are contemplated herein, probes based on the human DNA sequence of SEQ ID NO:1 or 3 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, *e.g.*, in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified.

Chromosome Mapping

All or a portion of the nucleic acids of SEQ ID NO:1 or more preferably SEQ ID NO:3, including oligonucleotides, can be used by those skilled in the art using well-known techniques to identify the human chromosome 17, and the specific locus thereof, that contains the DNA of Ig superfamily members. Useful techniques include, but are not limited to, using the sequence or portions, including oligonucleotides, as a probe in various well-known techniques such as radiation hybrid mapping (high resolution), in situ hybridization to

chromosome spreads (moderate resolution), and Southern blot hybridization to hybrid cell lines containing individual human chromosomes (low resolution).

For example, chromosomes can be mapped by radiation hybridization. First, PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids (http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/rhmap/genebridge4.html). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet (http://www-seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. The following web site provides additional information about radiation hybrid mapping: http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/07-97.INTRO.html).

Alternatively, PCR is performed using the Stanford G3 Radiation Hybrid Panel RH01.02 panel of 83 radiation hybrids (http://www-shgc.stanford.edu/About/index.html). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Stanford Human Genome Center site on the internet (http://www-shgc.stanford.edu/RH/rhserverformnew.html). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided.

Identifying Associated Diseases

As set forth below, SEQ ID NO:3, for example, has been mapped by PCR amplification to chromosome 17, next to markers SHGC-10198, SHGC-4026 and SHGC-36245 (Stanford Human Genome Center Rhserver). Thus, the nucleic acid sequences of SEQ ID NOS:1 or 3 or a fragment thereof can be used by one skilled in the art using well-known techniques to analyze abnormalities associated with gene mapping to chromosome 17. This enables one to distinguish conditions in which this marker is rearranged or deleted. In addition, nucleotides of SEQ ID NO:1 or 3 or a fragment thereof can be used as a positional marker to map other genes of unknown location.

The DNA may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, the genes corresponding to the nucleic acids of the invention. Disclosure herein of native nucleotide sequences permits the detection of defective genes, and the replacement thereof with normal genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparison of a native nucleotide sequence disclosed herein with that of a gene derived from a person suspected of harboring a defect in this gene.

Sense-Antisense

Other useful fragments of the nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of DNA (SEQ ID NOS:1 or 3). Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res. 48*:2659, 1988) and van der Krol et al. (*BioTechniques 6*:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block or inhibit protein expression by one of several means, including enhanced degradation of the mRNA by RNAseH, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in W091/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (*i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides

to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, lipofection, $CaPO_4$-mediated DNA transfection, electrophoration, or by using gene transfer vectors such as Epstein-Barr virus.

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

USE OF LSR POLYPEPTIDES AND FRAGMENTED POLYPEPTIDES

Uses include, but are not limited to, the following:

- Purifying proteins and measuring activity thereof
- Delivery Agents
- Therapeutic and Research Reagents
- Molecular weight and Isoelectric focusing markers
- Controls for peptide fragmentation
- Identification of unknown proteins
- Preparation of Antibodies

Purification Reagents

The polypeptide of the invention are also useful as a protein purification reagent. The polypeptides may be attached to a solid support material and used to purify the binding partner proteins by affinity chromatography. In particular embodiments, a polypeptide of the

instant invention (in any form described herein that is capable of binding the binding partner) is attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a polypeptide/Fc protein (as discussed above) is attached to Protein A- or Protein G- containing chromatography columns through interaction with the Fc moiety.

The polypeptide also finds use in purifying or identifying cells that express the binding partner on the cell surface. Polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with the polypeptides and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing the binding partner expressing cells are contacted with the solid phase having the polypeptides thereon. Cells expressing the binding partner on the cell surface bind to the fixed polypeptides, and unbound cells then are washed away.

Alternatively, the polypeptides can be conjugated to a detectable moiety, then incubated with cells to be tested for binding partner expression. After incubation, unbound labeled matter is removed and the presence or absence of the detectable moiety on the cells is determined.

In a further alternative, mixtures of cells suspected of containing cells expressing the binding partner are incubated with biotinylated polypeptides. Incubation periods are typically at least one hour in duration to ensure sufficient binding. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides binding of the desired cells to the beads. Procedures for using avidin-coated beads are known (see, Berenson et al., *J. Cell. Biochem. 10D*:239, 1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods.

Measuring Activity

Polypeptides also find use in measuring the biological activity of the binding partner protein in terms of their binding affinity. The polypeptides thus may be employed by those conducting "duality assurance" studies, *e.g.*, to monitor shelf life and stability of protein under different conditions. For example, the polypeptides may be employed in a binding affinity study to measure the biological activity of a binding partner protein that has been

stored at different temperatures, or produced in different cell types. The proteins also may be used to determine whether biological activity is retained after modification of a binding partner protein (*e.g.*, chemical modification, truncation, mutation, etc.). The binding affinity of the modified binding partner protein is compared to that of an unmodified binding partner protein to detect any adverse impact of the modifications on biological activity of the binding partner. The biological activity of a binding partner protein thus can be ascertained before it is used in a research study, for example.

Delivery Agents

The polypeptides also find use as carriers for delivering agents attached thereto to cells bearing the binding partner. The polypeptides thus can be used to deliver diagnostic or therapeutic agents to such cells (or to other cell types found to express the binding partner on the cell surface) in *in vitro* or *in vivo* procedures.

Detectable (diagnostic) and therapeutic agents that may be attached to a polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a calorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (*e.g.*, single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I, ^{131}I, ^{99m}Tc, ^{111}In, and ^{76}Br. Examples of radionuclides suitable for therapeutic use are ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}CU, and ^{67}CU.

Such agents may be attached to the polypeptide by any suitable conventional procedure. The polypeptide comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to polypeptides by using a suitable bifunctional chelating agent, for example.

Conjugates comprising polypeptides and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Therapeutic Agents

Polypeptides of the invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of the polypeptides. These polypeptides may be administered to a mammal afflicted with such a disorder.

The polypeptides may also be employed in inhibiting a biological activity of the binding partner, in *in vitro* or *in vivo* procedures. For example, a purified LSR polypeptide or LSR-fusion polypeptide may be used to inhibit binding of the binding partner to an endogenous cell surface polypeptide of the instant invention. Biological effects that result from the binding of the binding partner to endogenous receptors thus are inhibited.

In addition, LSR may be administered to a mammal to treat a binding partner-mediated disorder. Such binding partner-mediated disorders include conditions caused (directly or indirectly) or exacerbated by the binding partner.

Compositions of the present invention may contain a polypeptide in any form described herein, such as native proteins, variants, derivatives, oligomers, and biologically active fragments. Compositions comprising an effective amount of a polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. The polypeptides can be formulated according to known methods used to prepare pharmaceutically useful compositions. They can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (*e.g.*, saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (*e.g.*, thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions

will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance, and are thus chosen according to the intended application.

The compositions of the invention can be administered in any suitable manner, *e.g.*, topically, parenterally, or by inhalation. The term "parenteral" includes injection, *e.g.*, by subcutaneous, intravenous, or intramuscular routes, also including localized administration, *e.g.*, at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated, the patient's body weight, age, and general condition, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices.

Compositions comprising nucleic acids in physiologically acceptable formulations are also contemplated. DNA may be formulated for injection, for example.

Research Agents

Another use of the polypeptide of the present invention is as a research tool for studying the biological effects that result from inhibiting binding partner/LSR interactions on different cell types. Polypeptides also may be employed in *in vitro* assays for detecting binding partner or LSR or the interactions thereof.

Another embodiment of the invention relates to uses of LSR to study cell signal transduction. LSR could play a central role in immune responses which includes cellular signal transduction. As such, alterations in the expression and/or activation of LSR can have profound effects on a plethora of cellular processes. Expression of cloned LSR or of functionally inactive mutants of LSR can be used to identify the role a particular protein plays in mediating specific signaling events.

Cellular signaling often involves a molecular activation or inhibition cascade, during which a receptor propagates a ligand-receptor mediated signal by specifically activating intracellular kinases which phosphorylate target substrates or by activating intracellular phosphatases which trigger inhibitory signaling pathways. The protein kinase substrates can themselves be kinases which become activated following phosphorylation. Regardless of the nature of the substrate molecule(s), expressed functionally active versions of LSR and its binding partner can be used in assays such as the yeast 2-hybrid assay to identify what substrate(s) were recognized and activated by the polypeptides of the instant invention. As

such, these novel polypeptides can be used as reagents to identify novel molecules involved in signal transduction pathways.

Molecular Weight, Isoelectric Point Markers

The more preferred purified polypeptide of the invention (SEQ ID NO:4) has a calculated molecular weight of approximately 22,634 Daltons. Thus, where an intact protein is used, the use of these polypeptide molecular weight markers allows increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 15-20 kilodaltons. Where fragments are used, there is increased accuracy in determining molecular weight over the range of the molecular weights of the fragment.

The polypeptides of the present invention can be subjected to fragmentation into smaller peptides by chemical and enzymatic means, and the peptide fragments so produced can be used in the analysis of other proteins or polypeptides. For example, such peptide fragments can be used as peptide molecular weight markers, peptide isoelectric point markers, or in the analysis of the degree of peptide fragmentation. Thus, the invention also includes these polypeptides and peptide fragments, as well as kits to aid in the determination of the apparent molecular weight and isoelectric point of an unknown protein and kits to assess the degree of fragmentation of an unknown protein.

Although all methods of fragmentation are encompassed by the invention, chemical fragmentation is a preferred embodiment, and includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues (E. Gross, *Methods in Enz. 11*:238-255, 1967). This can further include additional steps, such as a carboxymethylation step to convert cysteine residues to an unreactive species.

Enzymatic fragmentation is another preferred embodiment, and includes the use of a protease such as Asparaginylendo-peptidase, Arginylendo-peptidase, *Achromobacter* protease I, Trypsin, *Staphlococcus aureus* V8 protease, Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to result in cleavage at specific amino acid residues. Asparaginylendo-peptidase can cleave specifically on the carboxyl side of the asparagine residues present within the polypeptides of the invention. Arginylendo-peptidase can cleave specifically on the carboxyl side of the arginine residues present within these polypeptides. *Achromobacter* protease I can cleave specifically on the carboxyl side of the lysine residues present within the polypeptides (Sakiyama and Nakat, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta 660*:44-50, 1981; T. Masaki et al.,

Biochim. Biophys. Acta 660:51-55, 1981). Trypsin can cleave specifically on the carboxyl side of the arginine and lysine residues present within polypeptides of the invention. Enzymatic fragmentation may also occur with a protease that cleaves at multiple amino acid residues. For example, *Staphlococcus aureus* V8 protease can cleave specifically on the carboxyl side of the aspartic and glutamic acid residues present within polypeptides (D.W. Cleveland, *J. Biol. Chem. 3*:1102-1106, 1977). Endoproteinase Asp-N can cleave specifically on the amino side of the asparagine residues present within polypeptides. Endoproteinase Lys-C can cleave specifically on the carboxyl side of the lysine residues present within polypeptides of the invention. Other enzymatic and chemical treatments can likewise be used to specifically fragment these polypeptides into a unique set of specific peptides.

Of course, the peptides and fragments of the polypeptides of the invention can also be produced by conventional recombinant processes and synthetic processes well known in the art. With regard to recombinant processes, the polypeptides and peptide fragments encompassed by invention can have variable molecular weights, depending upon the host cell in which they are expressed. Glycosylation of polypeptides and peptide fragments of the invention in various cell types can result in variations of the molecular weight of these pieces, depending upon the extent of modification. The size of these pieces can be most heterogeneous with fragments of polypeptide derived from the extracellular portion of the polypeptide. Consistent polypeptides and peptide fragments can be obtained by using polypeptides derived entirely from the transmembrane and cytoplasmic regions, pretreating with a glycosidase to remove glycosylation, or expressing the polypeptides in bacterial hosts.

The molecular weight of these polypeptides can also be varied by fusing additional peptide sequences to both the amino and carboxyl terminal ends of polypeptides of the invention. Fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention can be used to enhance expression of these polypeptides or aid in the purification of the protein. In addition, fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention will alter some, but usually not all, of the fragmented peptides of the polypeptides generated by enzymatic or chemical treatment. Of course, mutations can be introduced into polypeptides of the invention using routine and known techniques of molecular biology. For example, a mutation can be designed so as to eliminate a site of proteolytic cleavage by a specific enzyme or a site of cleavage by a specific chemically induced fragmentation procedure. The

elimination of the site will alter the peptide fingerprint of polypeptides of the invention upon fragmentation with the specific enzyme or chemical procedure.

The polypeptides and the resultant fragmented peptides can be analyzed by methods including sedimentation, electrophoresis, chromatography, and mass spectrometry to determine their molecular weights. Because the unique amino acid sequence of each piece specifies a molecular weight, these pieces can thereafter serve as molecular weight markers using such analysis techniques to assist in the determination of the molecular weight of an unknown protein, polypeptides or fragments thereof. The molecular weight markers of the invention serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of proteins that have similar apparent molecular weights and, consequently, allow increased accuracy in the determination of apparent molecular weight of proteins.

When the invention relates to the use of fragmented peptide molecular weight markers, those markers are preferably at least 10 amino acids in size. More preferably, these fragmented peptide molecular weight markers are between 10 and 100 amino acids in size. Even more preferable are fragmented peptide molecular weight markers between 10 and 50 amino acids in size and especially between 10 and 35 amino acids in size. Most preferable are fragmented peptide molecular weight markers between 10 and 20 amino acids in size.

Among the methods for determining molecular weight are sedimentation, gel electrophoresis, chromatography, and mass spectrometry. A particularly preferred embodiment is denaturing polyacrylamide gel electrophoresis (U.K. Laemmli, *Nature* 227:680-685, 1970). Conventionally, the method uses two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 6-20%. The ability to simultaneously resolve the marker and the sample under identical conditions allows for increased accuracy. It is understood, of course, that many different techniques can be used for the determination of the molecular weight of an unknown protein using polypeptides of the invention, and that this embodiment in no way limits the scope of the invention.

Each unglycosylated polypeptide or fragment thereof has a pI that is intrinsically determined by its unique amino acid sequence (which pI can be estimated by the skilled artisan using any of the computer programs designed to predict pI values currently available, calculated using any well-known amino acid pKa table, or measured empirically). Therefore these polypeptides and fragments thereof can serve as specific markers to assist in the determination of the isoelectric point of an unknown protein, polypeptide, or fragmented

peptide using techniques such as isoelectric focusing. These polypeptide or fragmented peptide markers serve particularly well for the estimation of apparent isoelectric points of unknown proteins that have apparent isoelectric points close to that of the polypeptide or fragmented peptide markers of the invention.

The technique of isoelectric focusing can be further combined with other techniques such as gel electrophoresis to simultaneously separate a protein on the basis of molecular weight and charge. The ability to simultaneously resolve these polypeptide or fragmented peptide markers and the unknown protein under identical conditions allows for increased accuracy in the determination of the apparent isoelectric point of the unknown protein. This is of particular interest in techniques, such as two dimensional electrophoresis (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77, Prentice Hall, 6d ed., 1991, where the nature of the procedure dictates that any markers should be resolved simultaneously with the unknown protein. In addition, with such methods, these polypeptides and fragmented peptides thereof can assist in the determination of both the isoelectric point and molecular weight of an unknown protein or fragmented peptide.

Polypeptides and fragmented peptides can be visualized using two different methods that allow a discrimination between the unknown protein and the molecular weight markers. In one embodiment, the polypeptide and fragmented peptide molecular weight markers of the invention can be visualized using antibodies generated against these markers and conventional immunoblotting techniques. This detection is performed under conventional conditions that do not result in the detection of the unknown protein. It is understood that it may not be possible to generate antibodies against all polypeptide fragments of the invention, since small peptides may not contain immunogenic epitopes. It is further understood that not all antibodies will work in this assay; however, those antibodies which are able to bind polypeptides and fragments of the invention can be readily determined using conventional techniques.

The unknown protein is also visualized by using a conventional staining procedure. The molar excess of unknown protein to polypeptide or fragmented peptide molecular weight markers of the invention is such that the conventional staining procedure predominantly detects the unknown protein. The level of these polypeptide or fragmented peptide molecular weight markers is such as to allow little or no detection of these markers by the conventional staining method. The preferred molar excess of unknown protein to polypeptide molecular weight markers of the invention is between 2 and 100,000 fold. More preferably, the

preferred molar excess of unknown protein to these polypeptide molecular weight markers is between 10 and 10,000 fold and especially between 100 and 1,000 fold.

It is understood of course that many techniques can be used for the determination and detection of molecular weight and isoelectric point of an unknown protein, polypeptides, and fragmented peptides thereof using these polypeptide molecular weight markers and peptide fragments thereof and that these embodiments in no way limit the scope of the invention.

In another embodiment, the analysis of the progressive fragmentation of the polypeptides of the invention into specific peptides (D.W. Cleveland et al., *J. Biol. Chem.* *252*:1102-1106, 1977), such as by altering the time or temperature of the fragmentation reaction, can be used as a control for the extent of cleavage of an unknown protein. For example, cleavage of the same amount of polypeptide and unknown protein under identical conditions can allow for a direct comparison of the extent of fragmentation. Conditions that result in the complete fragmentation of the polypeptide can also result in complete fragmentation of the unknown protein.

Finally, as to the kits that are encompassed by the invention, the constituents of such kits can be varied, but typically contain the polypeptide and fragmented peptide molecular weight markers. Also, such kits can contain the polypeptides wherein a site necessary for fragmentation has been removed. Furthermore, the kits can contain reagents for the specific cleavage of the polypeptide and the unknown protein by chemical or enzymatic cleavage. Kits can further contain antibodies directed against polypeptides or fragments thereof of the invention.

Identification of Unknown Proteins

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA 90*:5011-5015, 1993; D. Fenyo et al., *Electrophoresis 19*:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site:www.mann.embl-heiedelberg.de...deSearch/FR_PeptideSearch Form.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a

designated tolerance. The programs compare observed molecular weights to predicted peptide molecular weights derived from sequence databases to assist in determining the identity of the unknown protein.

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec. 5*:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem. 66*:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec. 11*:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbc.com:70/Lutefisk97.html), and the Protein Prospector, PeptideSearch and ProFound programs described above.

Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments, such as those of the instant invention, to a sequence database can aid in the identification of unknown proteins using mass spectrometry.

Antibodies

Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides *via* the antigen-binding sites of the antibody (as opposed to non-specific binding). Thus, the polypeptides, fragments, variants, fusion proteins, etc., as set forth above may be employed as "immunogens" in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C.A. Janeway, Jr. and P. Travers, *Immuno Biology 3*:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric hindrances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (C.A. Janeway, Jr. and P. Travers, *Immuno Biology 2*:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes may be identified by any of the methods known in the art.

Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies may be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the invention are also contemplated herein. Such hybridomas may be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. The monoclonal antibodies may be recovered by conventional techniques.

The monoclonal antibodies of the present invention include chimeric antibodies, *e.g.*, humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature 332*:323, 1988), Liu et al.

(*PNAS 84*:3439, 1987), Larrick et al. (*Bio/Technology 7*:934, 1989), and Winter and Harris (*TIPS 14*:139, May, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, U.S. Patent Nos. 5,569,825 and 5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein.

Antigen-binding fragments of the antibodies, which may be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

In one embodiment, the antibodies are specific for the polypeptides of the present invention and do not cross-react with other proteins. Screening procedures by which such antibodies may be identified are well known, and may involve immunoaffinity chromatography, for example.

Uses Thereof

The antibodies of the invention can be used in assays to detect the presence of the polypeptides or fragments of the invention, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

Those antibodies that additionally can block binding of the polypeptides of the invention to the binding partner may be used to inhibit a biological activity that results from such binding. Such blocking antibodies may be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of LSR to certain cells expressing the binding partner. Alternatively, blocking antibodies may be identified in assays for the ability to inhibit a biological effect that results from binding of their binding partner to target cells. Antibodies may be assayed for the ability to inhibit LSR-mediated or binding partner-mediated lysis, for example.

Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of the binding partner with cell surface (binding partner) receptor thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective in inhibiting a binding partner-mediated biological activity. Monoclonal antibodies are generally preferred

for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed.

Compositions comprising an antibody that is directed against LSR, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing LSR proteins.

Also provided herein are conjugates comprising a detectable (*e.g.*, diagnostic) or therapeutic agent, attached to the antibody. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

The polypeptides of the present invention may also be used in a screening assay to identify compounds and small molecules which inhibit (antagonize) or enhance (agonize) activation of the polypeptides of the instant invention. Thus, for example, polypeptides of the invention may be used to identify antagonists and agonists from cells, cell-free preparations, chemical libraries, and natural product mixtures. The antagonists and agonists may be natural or modified substrates, ligands, enzymes, receptors, etc. of the polypeptides of the instant invention, or may be structural or functional mimetics of the polypeptides. Potential antagonists of the polypeptides of the instant invention may include small molecules, peptides, and antibodies that bind to and occupy a binding site of the polypeptides, causing them to be unavailable to bind to their ligands and therefore preventing normal biological activity. Other potential antagonists are antisense molecules which may hybridize to mRNA in vivo and block translation of the mRNA into the polypeptides of the instant invention. Potential agonists include small molecules, peptides and antibodies which bind to the instant polypeptides and elicit the same or enhanced biological effects as those caused by the binding of the polypeptides of the instant invention.

Small molecule agonists and antagonists are usually less than 10K molecular weight and may possess a number of physiochemical and pharmacological properties that enhance cell penetration, resist degradation and prolong their physiological half-lives. (Gibbs, J., Pharmaceutical Research in Molecular Oncology, Cell, Vol. 79 (1994).) Antibodies, which include intact molecules as well as fragments such as Fab and F(ab')2 fragments, may be used to bind to and inhibit the polypeptides of the instant invention by blocking the commencement of a signaling cascade. It is preferable that the antibodies are humanized, and more preferable that the antibodies are human. The antibodies of the present invention may be prepared by any of a variety of well-known methods.

Specific screening methods are known in the art and many are extensively incorporated in high throughput test systems so that large numbers of test compounds can be screened within a short amount of time. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, cell based assays, etc. These assay formats are well known in the art (High Throughput Screening: The Discovery of Bioactive Substances, John P. Devlin (ed.), Marcel Dekker, New York, 1997, ISBN: 0-8247-0067-8; http://www.lab-robotics.org/; http://www.sbsonline.org/). The screening assays of the present invention are amenable to screening of chemical libraries and are suitable for the identification of small molecule drug candidates, antibodies, peptides and other antagonists and agonists.

One embodiment of a method for identifying molecules which antagonize (inhibit) or stimulate the polypeptides involves adding a candidate molecule to a medium which contains cells expressing the polypeptides of the instant invention; allowing the candidate molecule to bind the polypeptides of the instant invention; and observing the stimulation or inhibition of a functional response. The activity of the cells that were contacted with the candidate molecule may then be compared with the identical cells that were not contacted and agonists and antagonists of the polypeptides of the instant invention may be identified. The measurement of biological activity may be performed by a number of well-known methods such as measuring the amount of protein present (e.g. an ELISA) or of the protein's activity. A decrease in biological stimulation or activation would indicate an antagonist. An increase would indicate an agonist. Specifically, one embodiment of the instant invention includes agonists and antagonists of LSR.

Screening assays can further be designed to find molecules that mimic the biological activity of the polypeptides of the instant invention. Molecules that mimic the biological activity of a polypeptide may be useful for enhancing the biological activity of the polypeptide. To identify compounds for therapeutically active agents that mimic the biological activity of a polypeptide, a candidate molecule is added to a biological assay to determine its biological effects. The biological effects of the candidate molecule are then compared to those of the polypeptide.

Screening assays can even further be designed to find molecules (such as small molecules, peptides or antibodies) that mimic the biological activity of the natural LSR ligand. To identify compounds for therapeutically active agents that mimic the biological activity of the natural ligand of a polypeptide, it must first be determined whether a candidate

molecule binds to the polypeptide. A binding candidate molecule is then added to a biological assay to determine its biological effects. The biological effects of the candidate molecule are then compared to those of the natural ligand of the polypeptide.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference in their entirety.

EXAMPLE 1: Isolation of the Nucleic Acid

The human LSR sequences (SEQ ID NOS:1 and 3) were obtained by 5'RACE and PCR amplification using human leukocyte cDNA as a reaction template. The LSR sequences were amplified using a 5'RACE primer and a downstream primer whose sequence was derived from another Ig superfamily member (LIR-9). The LSR sequences were amplified due to the cross-hybridization of the LSR SEQ ID NOS:1 or 3 to the LIR-9 primer sequence.

EXAMPLE 2: Use of Purified LSR Polypeptides

Polypeptide-specific ELISA:

Serial dilutions of LSR-containing samples (in 50 mM $NaHCO_3$, brought to pH 9 with NaOH) are coated onto Linbro/Titertek 96 well flat bottom E.I.A. microtitration plates (ICN Biomedicals Inc., Aurora, OH) at 100:1/well. After incubation at 4°C for 16 hours, the wells are washed six times with 200:1 PBS containing 0.05% Tween-20 (PBS-Tween). The wells are then incubated with FLAG®-tagged LSR-binding partner at 1 mg/ml in PBS-Tween with 5% fetal calf serum (FCS) for 90 minutes (100:1 per well), followed by washing as above. Next, each well is incubated with the anti- FLAG® (monoclonal antibody M2 at 1 mg/ml in PBS-Tween containing 5% FCS for 90 minutes (100:1 per well), followed by washing as above. Subsequently, wells are incubated with a polyclonal goat anti-mIgGl-specific horseradish peroxidase-conjugated antibody (a 1:5000 dilution of the commercial stock in PBS-Tween containing 5% FCS) for 90 minutes (100:1 per well). The HRP-conjugated antibody is obtained from Southern Biotechnology Associates, Inc., Birmingham, Alabama. Wells then are washed six times, as above.

For development of the ELISA, a substrate mix [100:1 per well of a 1:1 premix of the TMB Peroxidase Substrate and Peroxidase Solution B (Kirkegaard Perry Laboratories, Gaithersburg, Maryland)] is added to the wells. After sufficient color reaction, the enzymatic

reaction is terminated by addition of 2 N H_2SO_4 (50:1 per well). Color intensity (indicating ligand receptor binding) is determined by measuring extinction at 450 nm on a V Max plate reader (Molecular Devices, Sunnyvale, CA).

EXAMPLE 3: Amino Acid Sequence

The amino acid sequences of LSR were determined by translation of the complete nucleotide sequences of SEQ ID NOS:1 and 3.

EXAMPLE 4: DNA and Amino Acid Sequences

The LSR nucleic acid sequences were determined by standard double stranded sequencing of the sequence obtained from 5'RACE and PCR amplifications as described in Example 1.

The nucleotide sequence of the isolated LSR and the amino acid sequence encoded thereby, are presented in SEQ ID NOs: 1, 2, 3 and 4. The sequence of the LSR DNA fragment isolated by PCR corresponds to nucleotides 1 to 852 of SEQ ID NO:1, which encode amino acids 1 to 201 of SEQ ID NO:2 and to nucleotides 1 to 852 of SEQ ID NO:3, which encode amino acids 1 to 201 of SEQ ID NO:4.

The amino acid sequences of SEQ ID NOS:2 and 4 bears significant homology to other known Ig ligand family members.

EXAMPLE 5: Monoclonal Antibodies That Bind Polypeptides of the Invention

This example illustrates a method for preparing monoclonal antibodies that bind LSR. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified LSR polypeptide or an immunogenic fragment thereof.

Purified LSR can be used to generate monoclonal antibodies immunoreactive therewith, using conventional techniques such as those described in U.S. Patent 4,411,993. Briefly, mice are immunized with LSR immunogen emulsified in complete Freund's adjuvant, and injected in amounts ranging from 10-100 μg subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional LSR emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for LSR antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay) or inhibition of LSR receptor binding.

Following detection of appropriate antibody titer, positive animals are provided one last intravenous injection of LSR in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, *e.g.*, NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified LSR by adaptations of the techniques disclosed in Engvall et al., (*Immunochem. 8*:871, 1971) and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol. 144*:4212, 1990). Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-LSR monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to Protein A or Protein G can also be used, as can affinity chromatography based upon binding to LSR.

EXAMPLE 6: Northern Blot Analysis

The tissue distribution of LSR is investigated by Northern blot analysis, as follows. An aliquot of a radiolabeled probe is added to two different human multiple tissue Northern blots (Clontech, Palo Alto, CA; Biochain, Palo Alto, CA). The blots are hybridized in 10X Denhardts, 50mM Tris pH 7.5, 900mM NaCl, 0.1% Na pyrophosphate, 1% SDS, 200 µg/mL salmon sperm DNA. Hybridization is conducted overnight at 63°C in 50% formamide as previously described (March et al., *Nature 315*:641-647, 1985). The blots are then washed with 2 x SSC, 0.1% SDS at 68°C for 30 minutes. The cells and tissues with the highest levels of LSR mRNA are determined by comparison to control probing with a β-actin-specific probe.

EXAMPLE 7: Chromosome Mapping

LSR was mapped using the Stanford G3 Radiation Hybrid panel RH01.02 (supplied by Research Genetics, Huntsville, AL). LSR PCR primers specific for the intron sequence

located between the sequences encoding the amino acids SHKR and amino acids NHYM in the spacer region were selected and used to screen a panel of 83 cell hybrids that contained different subsets of human chromosomes. The sequences of the primers used for mapping were the following ones: 5'-TCC CAC AAG AGG TGA GTC ATG TGG TTC-3' and 5'-TGT GAG CCA CCA GCC AAA GCC TGT GAC-3'. The data from the PCR amplifications were submitted to the Stanford Human Genome Center Rhserver (rhserver@shgc.stanford.edu), which mapped LSR to human chromosome 17 next to the markers SHGC-10198 (Lod score 21.58, distance 3 cRs), SHGC-4026 (Lod score 20.15, distance 6 cRs), and SHGC-36245 (Lod score 18.96, distance 6 cRs). Reference to the International RH Consortium Map (http://www.ncbi.nlm.nih.gov/genemap/) indicated that LSR maps to the interval on human chromosome 17 flanked by the D17S1352 (99.3cM) and D17S785 (104.7cM) markers, a region containing loci for the CMRF-35-H9 sequence (AF020314.1 reference in GenBank PRI division) and the thymidine kinase 1 sequence (K02581.1 reference in GenBank PRI division).

What is claimed is:

1. An isolated LSR nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence of SEQ ID NOS:1 or 3;

(b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence of SEQ ID NOS:2 or 4;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6 x SSC, at 42°C with washing conditions of 60°C, 0.5 x SSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:3; and

(e) an isolated nucleic acid molecule degenerate from SEQ ID NOS:1 or 3 as a result of the genetic code.

2. A recombinant vector that directs the expression of the nucleic acid molecule of claim 1.

3. An isolated polypeptide encoded by the nucleic acid molecule of claim 1.

4. An isolated polypeptide according to claim 3 in non-glycosylated form.

5. Isolated antibodies that bind to a polypeptide of claim 3.

6. Isolated antibodies according to claim 5, wherein the antibodies are monoclonal antibodies.

7. A host cell transfected or transduced with the vector of claim 2.

8. A method for the production of LSR polypeptide comprising culturing a host cell of claim 2 under conditions promoting expression, and recovering the polypeptide from the culture medium.

9. The method of claim 8, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

10. A method for the determination of the molecular weight of a sample protein comprising comparing molecular weight of a sample protein with the molecular weight of a polypeptide of claim 3;

wherein the comparison of molecular weights comprises application of the sample protein and polypeptide to an acrylamide gel, resolution of the sample protein and polypeptide using an electrical current, and application to the gel of a detection reagent, which stains the sample protein and polypeptide.

11. A kit for the determination of the molecular weights of peptide fragments of a sample protein comprising the following:

a vessel;

a polypeptide of claim 3;

at least one enzyme selected from the group consisting of Asparaginylendopeptidase, Arginylendopeptidase, *Achrombobacter* protease I, Trypsin, *Staphlococcus aureus* V8 protease, Endoproteinase Asp-N, and Endoproteinase Lys-C;

a mutated polypeptide from said polypeptide by *in vitro* mutagenesis, wherein a site of enzymatic cleavage by the selected enzyme has been removed; and

fragmented peptides derived from said peptide by enzymatic cleavage with the selected enzyme;

wherein said polypeptide and said sample protein are contacted with the selected protease; and wherein the protein, polypeptides, and fragmented peptides are visualized by application of the protein, polypeptides, and fragmented peptides to an acrylamide gel, resolution of the protein, polypeptides, and fragmented peptides using an electrical current, and application to the gel of a detection reagent, which stains the protein, polypeptides, and fragmented peptides.

12. An LSR polypeptide comprising an amino acid sequence selected from the group consisting of amino acids 19 through 201 of SEQ ID NO:4.

13. A method of screening a candidate molecule to identify its ability to inhibit (antagonize) or agonize a polypeptide encoded by a cDNA selected from the group consisting of SEQ ID NOS:1 or 3, said method comprising the steps of:

(a) culturing cells expressing the said polypeptide;

(b) changing the conditions of culture medium by adding said candidate molecule and allowing it to bind to the cells expressing the said polypeptide;

(c) determining the level of biological activity of said polypeptide complex formed after the addition of the said candidate to the said medium; and

(d) comparing the level of biological activity of step (c) with the level of biological activity that occurs in said medium in the absence of said candidate molecule;

wherein a decreased level of biological activity of step (c) as compared to the level of biological activity that occurs in said medium in the absence of said candidate molecule indicates an antagonist and an increased level of biological activity indicates an agonist.

14. An antagonist as identified by the method of claim 13.

15. The antagonist of claim 14, wherein said cDNA is the DNA sequence of SEQ ID NO:3.

16. An agonist as identified by the method of claim 13.

17. A method of screening a candidate molecule to identify its ability to mimic the biological activity of the polypeptide encoded by cDNA selected from the group consisting of SEQ ID NOS:1 or 3, said method comprising the steps of :

(a) adding said candidate molecule to a biological assay to determine its biological effects; and

(b) comparing said biological effects of said candidate molecule with the biological effects of the said polypeptide.

18. A method of screening a candidate molecule to identify its ability to mimic the biological activity of the natural ligand(s) of the polypeptide encoded by cDNA selected from the group consisting of SEQ ID NOS:1 or 3, said method comprising the steps of : .

(a) determining if said candidate molecule binds to said polypeptide or fragments thereof;

(b) adding said candidate molecule to a biological assay to determine its biological effects; and

(c) comparing said biological effects of said candidate molecule with the biological effects of the natural ligand(s) of the said polypeptide.

1 TGTCAATAAC CCCTCCTAGT TTGGGTGTTC CCATAGGCTG AGGGATGCCC
51 CTCCTGTACC CTTGGGGGAC ATAACAGTAT CTAATAAATT TCAAAGAGAA
101 ATGCCAGCGG CCGCTGAATT CTAGCTGGGA TCTGCATTTG CCACTGGTTG
151 CAGATCAGGC GGACGAGGAG CCGGGAAGGC AGAGCCATGT GGCTGCCCCC
201 TGCTCTGCTC CTTCTCAGCC TCTCAGGCTG TTTCTCCATC CAAGGCCCAG
251 AGTCTGTGAG AGCCCCAGAG CAGGGGTCCC TGACGGTTCA ATGCCACTAT
301 AAGCAAGGAT GGGAGACCTA CATTAAGTGG TGGTGCCGAG GGGTGCGCTG
351 GGATACATGC AAGATCCTCA TTGAAACCAG AGGGTCGGAG CAAGGAGAGA
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451 GTGACCATGG AGGGGCTCAC GCGAGATGAC GCAGATGTTT ACTGGTGTGG
501 GATTGAAAGA AGAGGACCTG ACCTTGGGAC TCAAGTGAAA GTGATTGTTG
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601 ATGGCAGTGT TCATCGGCTC CCACAAGAGG AACCACTACA TGCTCCTGGT
651 ATTTGTGAAG GTGCCCATCT TGCTCATCTT GGTCACTGCC ATCCTCTGGT
701 TGAAGGGGTC TCAGAGGGTC CCTGAGGAGC CAGGGGAACA GCCTATCTAC
751 ATGAACTTCT CCGAACCTCT GACTAAAGAC ATGGCCACTT AGAGAGATGG
801 ATCTGCAGAG CCTTCCTGCC CTGGCCACGT TTCCAGAAGA GACTCGGGCT
851 GT (SEQ ID NO:1)

FIGURE 1

1 MWLPPALLLL SLSGCFSIQG PESVRAPEQG SLTVQCHYKQ GWETYIKWWC

51 RGVRWDTCKI LIETRGSEQG EKSDRVSIKD NQKDRTFTVT MEGLTRDDAD

101 VYWCGIERRG PDLGTQVKVI VDPEGAASTT ASSPTNSNMA VFIGSHKRNH

151 YMLLVFVKVP ILLILVTAIL WLKGSQRVPE EPGEQPIYMN FSEPLTKDMA

201 T (SEQ ID NO:2)

FIGURE 2

1 TGTCAATAAC CCCTCCTAGT TTGGGTGTTC CCATAGGCTG AGGGATGCCC
51 CTCCTGTACC CTTGGGGGAC ATAACAGTAT CTAATAAATT TCAAAGAGAA
101 ATGCCAGCGG CCGCTGAATT CTAGCTGGGA TCTGCATTTG CCACTGGTTG
151 CAGATCAGGC GGACGAGGAG CCGGGAAGGC AGAGCCATGT GGCTGCCCCC
201 TGCTCTGCTC CTTCTCAGCC TCTCAGGCTG TTTCTCCATC CAAGGCCCAG
251 AGTCTGTGAG AGCCCCAGAG CAGGGGTCCC TGACGGTTCA ATGCCACTAT
301 AAGCAAGGAT GGGAGACCTA CATTAAGTGG TGGTGCCGAG GGGTGCGCTG
351 GGATACATGC AAGATCCTCA TTGAAACCAG AGGGTCGGAG CAAGGAGAGA
401 AGAGTGACCG TGTGTCCATC AAGGACAATC AGAAAGACCG CACGTTCACT
451 GTGACCATGG AGGGGCTCAC GCGAGATGAC GCAGATGTTT ACTGGTGTGG
501 GATTGAAAGA AGAGGACCTG ACCTTGGGAC TCAAGTGAAA GTGATTGTTG
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601 ATGGCAGTGT TCATCGGCTC CCACAAGAGG AACCACTACA TGCTCCTGGT
651 ATTTGTGAAG GTGCCCATCT TGCTCATCTT GGTCACTGCC ATCCTCTGGT
701 TGAAGGGGTC TCAGAGGGTC CCTGAGGAGC CAGGGGAACA GCCTATCTAC
751 ATGAACTTCT CCGAACCTCT GACTAAAGAC ATGGCCACTT AGAGAGATGG
801 ATCTGCAGAG CCTTCCTGCC CTGGCCACGT TTCCAGAAGA GACTCGGGCT
851 GT (SEQ ID NO:3)

FIGURE 3

1 MWLPPALLLL SLSGCFSIQG PESVRAPEQG SLTVQCHYKQ GWETYIKWWC
51 RGVRWDTCKI LIETRGSEQG EKSDRVSIKD NQKDRTFTVT MEGLTRDDAD
101 VYWCGIERRG PDLGTQVKVI VDPEGAASTT ASSPTNSNMA VFIGSHKRNH
151 YMLLVFVKVP ILLILVTAIL WLKGSQRVPE EPGEQPIYMN FSEPLTKDMA
201 T(SEQ ID NO:4)

FIGURE 4

SEQUENCE LISTING

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Borges, Luis
Cosman, David

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HUMAN LEUKOCYTES

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ctagctggga tctgcatttg ccactggttg cagatcaggc ggacgaggag ccgggaaggc 180

agagcc atg tgg ctg ccc cct gct ctg ctc ctt ctc agc ctc tca ggc 228
Met Trp Leu Pro Pro Ala Leu Leu Leu Leu Ser Leu Ser Gly
1 5 10

tgt ttc tcc atc caa ggc cca gag tct gtg aga gcc cca gag cag ggg 276
Cys Phe Ser Ile Gln Gly Pro Glu Ser Val Arg Ala Pro Glu Gln Gly
15 20 25 30

tcc ctg acg gtt caa tgc cac tat aag caa gga tgg gag acc tac att 324
Ser Leu Thr Val Gln Cys His Tyr Lys Gln Gly Trp Glu Thr Tyr Ile
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145 150 155

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INTERNATIONAL SEARCH REPORT

Internat. .l Application No
PCT/US 00/09928

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C07K16/28 G01N33/68 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 897 981 A (SMITHKLINE BEECHAM PHILADELPHIA (US) WU S; SWEET RW; TRUNEH A HURLE MR) 24 February 1999 (1999-02-24) the whole document -----	1-9,12, 13,17,18

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2000	08/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Macchia, G

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 14-16

Present claims 14-16 refer to an antagonist and agonist of the polypeptide disclosed, without giving a true technical characterization. Moreover no specific compounds are defined in the application. In consequence, the scope of said claim is ambiguous and vague, anhd their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/09928

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0897981 A	24-02-1999	US 6020161 A	01-02-2000
		CA 2240631 A	19-02-1999
		JP 11225774 A	24-08-1999